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Meiothermus ruber Genome Analysis Project

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***TMrub_1675, Mrub_1676, Mrub_1677, and Mrub_1679* genes are orthologs of *b_3458, b_3457, b_3456, and b_3454* genes in *E. coli*, respectively, coding for ABC transporters. *Mrub_1678 and b_3455*, though perform similar tasks, are not orthologous.**

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Introduction

Meiothermus Ruber: What is it? And why study it?

Meiothermus Ruber (M.ruber), originally named *Thermus ruber*, was named from Greek origins, 'meion', meaning 'lesser' and 'thermos', meaning 'hot'. The species name, 'ruber', is named after the pigment color of M.ruber, a bright red (Tindall *et al.*, 2010). However,, the species is heterogenous (Lapage et al., 1952), in respect to the pigment color , and the other species in the genus display different pigmentation colors (Tenreiro et al., 1995), ranging from a pale yellow to a deep orange (Tindall *et al.*, 2010).

M.ruber, originally named *Thermus ruber* (Skerman)(Lapage et al., 1952), is a gram negative, aerobic, rod shaped bacteria.The species was first found in and around Russian hot springs by Loginova (Loginova et al., 1987), where they thrive in their optimal temperature of about 60°C (Loginova et al., 1987). Interestingly enough,

however, the heat loving bacteria is not very well researched (Albuquerque et al ., 2009). There are only 28 publications for *M.ruber* (Scott et al., 2015) where as there are over 30,000 for *E.coli* and *Salmonella*. However, the importance of this study comes from the Joint Genome Institute and the Genomic Encyclopedia of Bacteria and Archaea (GEBA) project . The project aims to study lesser understood organisms (Phylogenetic Diversity et al ., 2015) because they may reveal processes or variants of processes that are not present in other well known organisms. Thus, the GEBA project aims to look into organisms that are often overlooked.

E. Coli* as a control to study *M.Ruber

This project used a well known model organism, *E.coli* as a control and a point of comparison/contraction to better understand *M.ruber*. Model organisms, such as *E.coli*, have shown to grow very well in lab and replicate very rapidly and are not too difficult to maintain, which makes them good model organisms. These factors are some of the reasons why *E.coli* has been studied so extensively. However, the reason *E.coli* was chosen as the control for this project was that a BLAST search revealed that *E.coli*'s *Pro C* protein sequence was very similar to that of *M.ruber*. It was later proved that *M.ruber_1345* gene was an ortholog of the *b_0386* gene of *E.coli*, which coded for Pyrroline-5-carboxylate reductase. To further investigate the orthology between *E.coli* and *M.ruber* we will look into a variety of branched chain amino acid ABC transporters.

ABC Transporter Proteins

Gases, polar molecules and small nonpolar molecules can cross the plasma membrane relatively easily. However, most substrates do not fall into these three categories and require a protein to cross the plasma membrane. ABC transporters are just some of the proteins cells use to transport molecules that fall outside of the three listed categories. Considering that most substrates are not gases, polar molecules or small nonpolar molecules, it is easy to make sense of the fact that a large portion of most genomes are dedicated to creating proteins that are transporters. ABC transporters, in specific, are a special type of transmembrane protein comprised of two nucleotide-binding domains (NBD), which initially bind to a substrate outside the cell; and two transmembrane domains (TMD), which are segments that cross the plasma membranes (Wilkins, et al., 2015). ATP is hydrolyzed on the NBD which causes a conformational change in the TMDs and allows for the solute of interest to enter the cell (Kaiyani et al., 2016).

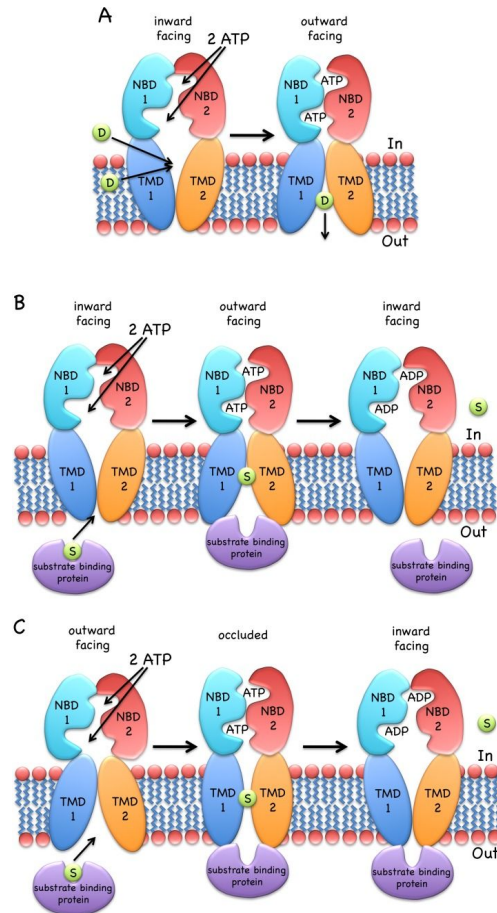


Figure 1 shows how to solute will bind to the receptor of NBD, which will allow for ATP to bind. ATP then hydrolyzed, causes the conformational change in the TMDs and the solute enters. Once ADP+Pi (the product of ATP hydrolysis) is released, the protein 'resets' (Kaiyani et al., 2016).

Bioinformatics:

The bioinformatics programs used in this project are very important to use in many applications among many biological practices. From the simple protein BLAST (Madden *et al.*, 2002), to the more indepth analyzation of genes, bioinformatics can give information in a wide variety of specific genes, pathways, and gene sequences. Access to these programs gives the science community immensely knowledgeable databases that is easily accessible to in research and projects such as this to save time. As science continues to make advances, bioinformatics tools will advance along with discoveries and be even more accessible to scientists and researchers.

Purpose/Hypothesis:

The purpose of this project is to determine if *Mrub_1675*, *Mrub_1676*, *Mrub_1677*, *Mrub_1678*, and *Mrub_1679* genes are orthologs of *b_3458*, *b_3457*, *b_3456*, *b_3455*, and *b_3454* genes in *E. coli*, respectively. We determined this by using multiple bioinformatics tools to show us the similarities and differences between the genes to tell if they are indeed orthologs of eachother. An important value to understand is the E-values. The E-values that come from the programs help to determine the significance of of the results. If the E-values generated are high values, the sequences are more likely to be aligned versus low E-values which indicate that the sequence is significantly different and therefore could be orthologs. To begin our original hypothesis, the *M. ruber* genes were BLASTed (Madden *et al.*, 2002) against *E. coli* and the *E. coli* genes were BLASTed (Madden *et al.*, 2002) against *M. ruber* and low E-values were obtained.

Since low E-values were generated, we can hypothesize that the genes are orthologs of each other, but further bioinformatic analysis will further confirm the hypothesis.

Methods

In this gene annotation project, the GENI-ACT gene annotation instructions (Scott *et al.*, 2016) were followed as well as the addition of 15 BLAST (Madden *et al.*, 2002) hits in T-coffee (Notredame *et al.*, 2000), EcoCyc (Keseler *et al.*, 2013), and colored by KEGG (Kanehisa *et al.*, 2016) were supplementally used to generate bioinformatics data. To start the research, the *M. ruber* genes were BLASTed against *E. coli* and the *E. coli* genes were BLASTed against *M. ruber*. Once the genes were BLASTed, the GENI-ACT site instructions (Scott *et al.*, 2016) were followed and the bioinformatics tools were used to generate data to help confirm the orthologs. Using the T-coffee program (Notredame *et al.*, 2000), we used 15 BLAST (Madden *et al.*, 2002) hits for each gene. Instead of using MetaCyc for the ABC transporter pathway information, we used the EcoCyc (Keseler *et al.*, 2013) program. To be able to visualize the genes upstream and downstream of the genes in question, we used colored by KEGG (Kanehisa *et al.*, 2016) to colorfully see the genes involved in ABC transportation. The KEGG (Kanehisa *et al.*, 2016) genome pathway program was used to visualize the pathway of the genes and to help identify what mechanism of action the pathway was involved in. KEGG (Kanehisa *et al.*, 2016) was also used to identify locus tags, DNA coordinates, nucleotide sequences, and amino acid sequences of the genes in question. Next, EcoCyc (Keseler *et al.*, 2013), was used to visualize the pathway of the genes in a form

of their functionality in ABC transportation. EcoCyc (Keseler *et al.*, 2013) was also used to visualize the operon map for *E. coli* pathway. NCBI BLAST (Madden *et al.*, 2002) was used to show pairwise alignment between the genes and *M. ruber* and *E. coli*, the resulting bit score and E-values were used to show the orthologs of the genes. Integrated Microbial Genomes and Microbiomes (IMG/M) (Markowitz *et al.*, 2012) program was used for proposed DNA coordinates as well as identifying Shine-Delgarno sequences to help aid in identifying if the original start codon was called correctly. IMG/M (Markowitz *et al.*, 2012) was also used towards the end of the project to display a chromosome map of our genes in question and were able to analyze upstream and downstream genes. Another bioinformatics tool that was used was T-coffee (Notredame *et al.*, 2000) and it was used to find sequence alignments in different bacterias and give the possibility to align all of the different sequences. Being able to align multiple sequences, it allows the visualization of seeing if the start codon was called correctly. Another bioinformatics tool that was helpful in calling the original start codon was Weblogo (Crooks *et al.*, 2016). Weblogo (Crooks *et al.*, 2016) was a great visual tool to show the highly conserved amino acids that were in the sequences that helped to further confirm the start codon was called correctly. TMHMM (Krogh *et al.*, 2001), SignalP (Kall *et al.*, 2004) , LipoP (Juncker *et al.*, 2016) , PSORT-B (Yu *et al.*, 2010), and Phobius (Kall *et al.*, 2007) were all used for the visualization and confirmation of transmembrane proteins and to locate where the proteins reside. NCBI (Madden *et al.*, 2002) bioinformatics program was also used to get CDD results and COG information to further confirm the genes roles with accompanying bit scores and E-values. TIGRfam

(Haft *et al.*, 2001) and Pfam (Finn *et al.*, 2014, Finn *et al.*, 2016) programs were used to confirm the genes roles with accompanying bit scores and E-values as well with the use of the sequence family classifications, pairwise alignments, and HMM logos. Another important bioinformatics tool was PDB (Berman *et al.*, 2000) and it showed protein structures that were involved with the genes in our pathways. It also showed pairwise alignments, bit score, E-value, and other research that had been done.

Results

Table 1:

Bioinformatics tool used	<i>E. coli</i> b_3454 (<i>liv F</i>)	<i>Mrub</i> _1679 (<i>liv F</i>)
BLAST <i>E.coli</i> against <i>M. ruber</i>	Score: 241 bits E-value: 2e-81	
CDD Data (COG Category)	COG0410 ABC-type branched-chain amino acid transport system	<u>COG0410</u> ABC-type branched-chain amino acid transport system
Cellular Localization	Cytoplasm	Cytoplasm
TIGRfam- protein family	TIGR03410 urea ABC transporter E-value: 4.7e-60	<u>TIGR03410</u> urea ABC transporter, urea binding protein 2.24e-76
Pfam- protein family	PF00005: ATP-binding domain of ABC transporters <hr/> E-Value: 3.3e-33	PF00005: ATP-binding domain of ABC transporters E-value: 2.42e-46

Protein Database	Crystal structure of an ABC-type branched-chain amino acid transporter (RPA4397) from <i>Rhodopseudomonas palustris</i> CGA009 at 1.50 Å resolution E-value 0.0	ABC transporter 6.38e-36
KEGG pathway map	Branched Chain amino acid transport	Branched Chain amino acid transport

Table 1: The table above compares *E. coli b_3454 (liv F)* and *Mrub_1679 (liv F)* with the bioinformatics tools used. The bioinformatics tools used include NCBI BLAST (Madden *et al.*, 2002), IMG/M (Markowitz *et al.*, 2012), TIGRfam (Haft *et al.*, 2001), Pfam (Finn *et al.*, 2014, Finn *et al.*, 2016), PDB (Berman *et al.*, 2000), and KEGG (Kanehisa *et al.*, 2016).

Table 2:

Bioinformatics tool used	<i>E. coli b_3455 (liv G)</i>	<i>Mrub_1678 (liv G)</i>
BLAST <i>E.coli</i> against <i>M. ruber</i>	Score: 248 bits E-value: 7e-84	
CDD Data (COG Category)	COG0411 ABC-type branched-chain amino acid transport system	<u>COG4177</u> ABC-type branched-chain amino acid transport system, permease component
Cellular Localization	Cytoplasm	cytoplasm
TIGRfam- protein family	TIGR03411 urea ABC transporter	<u>TIGR03408</u>

	3.7e-65	
Pfam- protein family	PF00005: ATP-binding domain of ABC transporters <hr/> E-Value: 3.1e-32	PF00001: urea ABC transporter, permease protein UrtC 2.51e-18
Protein Database	Crystal structure of an ABC-type branched-chain amino acid transporter (RPA4397) from <i>Rhodopseudomonas palustris</i> CGA009 at 1.50 Å resolution E-value 0.0	Branched-chain amino acid transport system (CL0142) 4.56e-13
KEGG pathway map	ABC Transporter	ABC transporter

Table 2: The table above compares *E. coli* *b_3455* (*liv G*) and *Mrub_1678* (*liv G*) with the bioinformatics tools used. The bioinformatics tools used include NCBI BLAST (Madden *et al.*, 2002), IMG/M (Markowitz *et al.*, 2012), TIGRfam (Haft *et al.*, 2001), Pfam (Finn *et al.*, 2014, Finn *et al.*, 2016), PDB (Berman *et al.*, 2000), and KEGG (Kanehisa *et al.*, 2016).

Table 3:

Bioinformatics tool used	<i>E. coli</i> b_3456 (<i>liv M</i>)	<i>Mrub</i> _1677 (<i>liv M</i>)
BLAST <i>E.coli</i> against <i>M. ruber</i>	Score: 184 bits E-value: 5e-54	
CDD Data (COG Category)	COG4177 ABC-type branched-chain amino acid transport system	<u>COG4177</u> ABC-type branched-chain amino acid transport system, permease component
Cellular Localization	Transmembrane	Transmembrane
TIGRfam- protein family	TIGR03410 urea ABC transporter 1e-11	<u>TIGR03408</u> urea ABC transporter, permease protein UrtC
Pfam- protein family	PF02653: Branched-chain amino acid transport system / permease component E-Value: 9e-60	<u>Pf02653</u> Branched-chain amino acid transport system / permease component 4.56e-13
Protein Database	Crystal structure of an ABC-type branched-chain amino acid transporter (RPA4397) from <i>Rhodopseudomonas palustris</i> CGA009 at 1.50 Å resolution E-value 0.0	No PDB results
KEGG pathway map	ABC Transporter	ABC transporter

Table 3: The table above compares *E. coli* b_3456 (*liv M*) and *Mrub*_1677 (*liv M*) with the bioinformatics tools used. The bioinformatics tools used include NCBI BLAST

(Madden *et al.*, 2002), IMG/M (Markowitz *et al.*, 2012), TIGRfam (Haft *et al.*, 2001), Pfam (Finn *et al.*, 2014, Finn *et al.*, 2016), PDB (Berman *et al.*, 2000), and KEGG (Kanehisa *et al.*, 2016).

Table 4:

Bioinformatics tool used	<i>E. coli</i> b_3457 (<i>liv H</i>)	<i>Mrub_1676</i> (<i>liv H</i>)
BLAST <i>E.coli</i> against <i>M. ruber</i>	Score: 201 bits E-value: 7e-64	
CDD Data (COG Category)	COG0559 ABC-type branched-chain amino acid transport system	COG0559 Branched-chain amino acid ABC-type transport system,
Cellular Localization	Transmembrane	Transmembrane
TIGRfam- protein family	TIGR03410 urea ABC transporter 3.7e-8	TIGR03409 Urea ABC transporter
Pfam- protein family	PF02653:Branched-chain amino acid transport system / permease component <hr/> E-Value: 6.7e-71	<u>pfam02653</u> Branched-chain amino acid transport system / permease component <hr/> 6.20e-18
Protein Database	Crystal structure of an ABC-type branched-chain amino acid transporter (RPA4397) from <i>Rhodopseudomonas palustris</i> CGA009 at 1.50 Å resolution E-value 0.0	<u>Solution Structure of PHAX-RBD in complex with ssRNA (2XC7)</u>

KEGG pathway map	ABC Transporter	ABC transporter
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Table 4: The table above compares *E. coli b_3457 (liv H)* and *Mrub_1676 (liv H)* with the bioinformatics tools used. The bioinformatics tools used include NCBI BLAST (Madden *et al.*, 2002), IMG/M (Markowitz *et al.*, 2012), TIGRfam (Haft *et al.*, 2001), Pfam (Finn *et al.*, 2014, Finn *et al.*, 2016), PDB (Berman *et al.*, 2000), and KEGG (Kanehisa *et al.*, 2016).

Table 5:

Bioinformatics tool used	<i>E. coli b_3458 (liv K)</i>	<i>Mrub_1675 (liv K)</i>
BLAST <i>E.coli</i> against <i>M. ruber</i>	Score: 173 bits E-value: 8e-52	
CDD Data (COG Category)	COG0683 ABC-type branched-chain amino acid transport system	COG0683 ABC-type branched-chain amino acid transport system
Cellular Localization	Cytoplasm	Cytoplasm
TIGRfam- protein family	TIGR03407 urea ABC transporter -166.5	Tigr03407 urea ABC transporter, urea binding protein
Pfam- protein family	PF13458: Periplasmic binding protein <hr/> E-Value: 1.7e-61	PF13458: Periplasmic binding protein E-value: 2.42e-46

Protein Database	Crystal structure of an ABC-type branched-chain amino acid transporter (RPA4397) from <i>Rhodopseudomonas palustris</i> CGA009 at 1.50 Å resolution	Extracellular ligand binding receptor from <i>Desulfohalobium retbaense</i> DSM5692
	E-value 0.0	E-value: 8.88e-16
KEGG pathway map	ABC Transporter	Branched Chain amino acid transport

Table 5: The table above compares *E. coli* *b_3458* (*liv K*) and *Mrub_1675* (*liv K*) with the bioinformatics tools used. The bioinformatics tools used include NCBI BLAST (Madden *et al.*, 2002), IMG/M (Markowitz *et al.*, 2012), TIGRfam (Haft *et al.*, 2001), Pfam (Finn *et al.*, 2014, Finn *et al.*, 2016), PDB (Berman *et al.*, 2000), and KEGG (Kanehisa *et al.*, 2016).

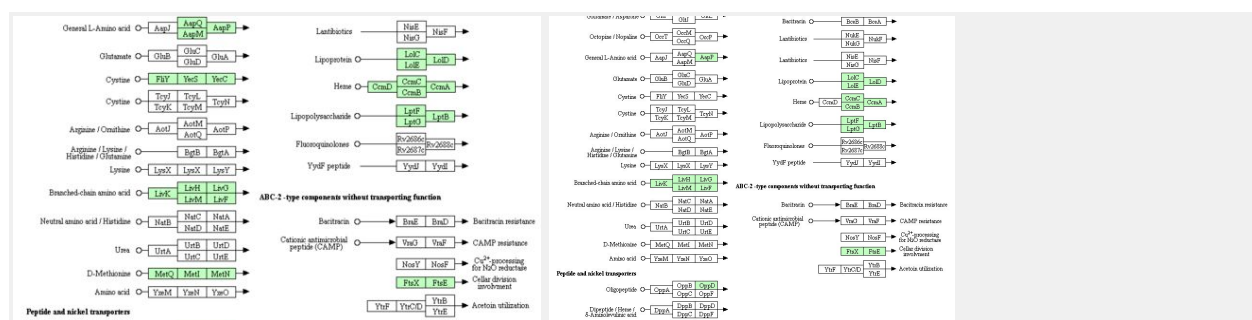


Figure 2: The pathway maps of *E. coli* (left) and *M. ruber* (right) are shown. Both have the branched chain amino acids.

E. coli* *b_3454* / *Mrub_1679

Table 1 summarizes the results generated from a variety of bioinformatics tools. The BLAST data shows that the e-value is $2e-81$, which is significantly below the .001 cutoff and suggests that the sequence similarity is likely not by chance. The CDD data, also generated the same COG number, TIGRfam and Pfam all generated the same protein number as well, with very low e-values, which further shows that the two genes are not similar by chance. TMH, SignalP, LipoP, and PSORT-B all suggested that the proteins are found in the in the cytoplasm of the cell. They both also lack a cleavage site. The two proteins are, by the evidence provided, likely orthologous ABC transporters.

Figure 3 shows the BLAST results, when *E.coli* BLASTed against *M.ruber*.

ABC transporter ATP-binding protein [Meiothermus ruber]
Sequence ID: [WP_013014902.1](#) Length: 237 Number of Matches: 1
[▶ See 2 more title\(s\)](#)

Range 1: 3 to 236 [GenPept](#) [Graphics](#) [▼](#) Next Match [▲](#) Previous Match

Score	Expect	Method	Identities	Positives	Gaps
241 bits(614)	2e-81	Compositional matrix adjust.	122/234(52%)	159/234(67%)	1/234(0%)
Query 5	MLSFDKVSAHYGKIQALHEVSLHINQGEIVTLIGANGAGKTTLLGTLCGDPRATSGRIVF	64			
	+L + +YG I AL VSL + +GEIVTLIGANGAGK+T L T+ G + +G +++				
Sbjct 3	LLEVKDIHTYYGHIHALKGVSLTVEEGEIVTLIGANGAGKSTTLRTISGMNPKRKGEVLY	62			
Query 65	DDKDITDWQTAKIMREAVAIVPEGRRVFSRMTVEENLAMGGFFAERDQF-QERIKWVYEL	123			
	I KI+ + VPEGRR+F RMTVEENL MGGF + QER + + L				
Sbjct 63	QGSPHKLPAADKIVGLGIGHVPEGRRIFRMTVEENLDMGGFLIRDPKVVQERKEQAFTL	122			
Query 124	FPRLHERRIQRAGTMSGGEQQMLAIGRALMSNPRLLLLDEPSLGLAPTIIQQIFDTIEQL	183			
	FPRL ERR Q+ GT+SGGEQQMLAIGRALM +P+LLL+DEPS+GLAP+++ IF+ I++L				
Sbjct 123	FPRLAERRNQKGGTMSGGEQQMLAIGRALMQDPKLLLMDEPSHGLAPVLVDFFIFEIIQKL	182			
Query 184	REQGMTIFLVEQNAQALKLADRGVYVLENGHVLSDTGDALLANEAVRSAYLGG	237			
	+QG TI LVEQNA AL++A RGYVL+ G + +S L A ++ AYLGG				
Sbjct 183	NQQGKTILLVEQNAQLALQIAHRGYVLQTGQLTMSGPAKELAAARPEIQEAYLGG	236			

Figure 3: BLAST results from *E.coli* *b_3454* BLASTed against *Mrub_1679* (Madden et al., 2002)

E. coli *b_3455* / *Mrub_1678*

Table 2 summarizes the results generated from a variety of bioinformatics tools. The BLAST data generated shows an e-value of $7e-84$, which suggests that the two proteins

are not similar by chance. However, the proteins generated 2 different COG numbers, 2 different TIGR numbers and two different Pfam numbers. Though the two proteins have shown to be found in the same location (TMH, SignalP, LipoP, and PSORT-B) and serve similar functions, they do not seem to be orthologous. This is not a shock, because the proteins are from different phyla, and more than likely found different ways to carry out similar functions. Figure 4 shows the BLAST results.

Range 1: 1 to 252 [GenPept](#) [Graphics](#) [▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
248 bits(634)	7e-84	Compositional matrix adjust.	124/252(49%)	168/252(66%)	0/252(0%)
Query 1	MSQPLLSVNGLMRFGGLAVNNVNLELYPQEIIVSLIGPNGAGKTTVFNCLTGFYKPTGG	60			
	MS+ L V +FGGL+AVNNV+L++ P+EI S+IGPNGAGKTT FN LTG YKP G				
Sbjct 1	MSELALDVQNAKKFGGLVAVNNVSLQVRPKEIFSVIGPNGAGKTTFFNLLTGIVKPDG	60			
Query 61	TILLRDQHLEGLPGQIARMGVVRTFQHVRLFREMTVIENLLVAQHQLKTLGFSGLLKT	120			
	++ + + G ++AR G+ RTFQ++RLF+ MTV+EN+LV H LL T				
Sbjct 61	KVVFFGKDITGYSPDKVARTGIGRTFQNIIRLFKAMTVLENVLVGHHSLSLTHQSYLDVLLHT	120			
Query 121	PSFRRAQSEALDRAATWLERIGLLEHANRQASNLAYGDQRRLEIARCMVTQPEILMLDEP	180			
	P F ++ +A RA L + L + A AS L+YG+QRRLEIAR + +P +L LDEP				
Sbjct 121	PRFHASERKAKARAMELLAYMNLDKRAEELASGLSYGEQRRLEIARALALEPRLFLDEP	180			
Query 181	AAGLNPKETKELDELIAELRNHHNTTILLIEHDMKLVMGISDRIYVVNQGTPLANGTPEQ	240			
	AAG+N +ET++L + +LR+ TI+LIEHDM +VM ISDRI V+ G+ +A G P +				
Sbjct 181	AAGMNEQETEDLKVRVRKLRDDGLTIVLIEHDMAMVMSISDRIAVLEVGSKTAEGLPAE	240			
Query 241	IRNNDPDIRAYL	252			
	IR+NP VI AYL				
Sbjct 241	IRSNPRVIEAYL	252			

Figure 4: BLAST results from *E.coli* b_3455 BLASTed against *Mrub_1678* (Madden et al., 2002)

E. coli* b_3456 / *Mrub_1677

Table 3 summarizes the results generated from a variety of bioinformatics tools. The BLAST data shows that the e-value is 5-e54, which is significantly below the .001 cutoff and suggests that the sequence similarity is likely not by chance. The CDD data, also generated the same COG number, TIGRfam and Pfam all generated the same protein number as well, with very low e-values, which further shows that the two genes are not similar by chance. TMH, SignalP, LipoP, and PSORT-B all suggested that the proteins are found embedded in the plasma membrane of the cell. They both also lack a

cleavage site. The two proteins are, by the evidence provided, likely orthologous ABC transporters. Figure 5 shows the BLAST results.

Range 1: 86 to 473 [GenPept](#) [Graphics](#) [▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
184 bits(466)	5e-54	Compositional matrix adjust.	146/397(37%)	209/397(52%)	58/397(14%)
Query 77	FILPAIDGSTVKQKFLVALLVLAVAWPFMVSRGTVDIATLTMIIYIILGLGLNVVVGSLG				136
Sbjct 86	F+LP + ST+ + A+L +AV + + ++ I+ L LGLNVVVG +G				
	FLLPNL--STLVRVALGAAILFIAVPIAGLTNSFLFELGIQIGIFAALALGLNVVVGQAG				143
Query 137	LLVLGYGGFYAIGAYTFALLNHYYGLGFWTCLPIAGL-----MAAAAGFLL				182
Sbjct 144	LL LG+ F+AIGAYT+ + F P GL AA G L+				
	LLDLGFAAFFAIGAYTWGIFGSPQAAQFIPGYPSEGLPGNYLYLFMALAVITAAITGVLI				203
Query 183	GFPVLRRLRGDYLAIVTLGFGEIVRILLNTE----ITGGPNGISQIPKPTLFGL-EFSR				237
Sbjct 204	G P LRLRGDYLAIVTLG GE+VR+ NN + IT GP GI+ + +P + L EF R				
	GLPALRLRGDYLAIVTLGLGEVVRVFA-NNLDKPLNITNGPQGITPVNRPEVGPLTEFLR				262
Query 238	TAREGGWDTFSNFFGLKYD-PSDRVIFLYLVALLLVLSLFVINRLLRMPLGRAWEALRE				296
Sbjct 263	+G D P F YL+ L+++ + + V RL GRAW A+RE				
	AIGA-----ERLYGRPIDEPIAYAFFFYLLVLVVGIVVLVNIIRLANSRFGRAWVAIRE				316
Query 297	DEIACRSGLSPRIKLTAFITISAAFAGFAGTLFAARQGFVSPESFTFAESAFVLAIVVL				356
Sbjct 317	DEIA +++G+ KL AF AAF+G G +FAA+Q FVSPESFT S +LA V+L				
	DEIAAKAMGIPLLPKLLAFATGAAFSGAMGAIFAAKQTFVSPESFTLQASINILAFVIL				376
Query 357	GGMGS-QFAVILAAAILLVVSRELMRDFNE-----YSM				387
Sbjct 377	GGMGS AV+ AA + V++ +++DF++ Y				
	GGMGSIGGAVVGAAAVTVLNIGILKDFSLLNTWRQTGVITILGYNMANLPPQLNPAKYER				436
Query 388	LMLGGLHVLMMIWRPQGLLPMTRPQLKLNKAAGEQ		424		
Sbjct 437	L+ G +++LMMI+RP+GL+P R + +L+ + ++				
	LVFGLILILMMIFRPEGLIPEQRHRAELQEARQEAKE		473		

Figure 5: BLAST results from *E.coli* b_3456 BLASTed against *Mrub_1677* (Madden et al., 2002)

E. coli* b_3457 / *Mrub_1676

Table 4 summarizes the results generated from a variety of bioinformatics tools. The BLAST data shows that the e-value is 7e-64, which is significantly below the .001 cutoff and suggests that the sequence similarity is likely not by chance. The CDD data, also generated the same COG number, TIGRfam and Pfam all generated the same protein number as well, with very low e-values, which further shows that the two genes are not similar by chance. TMH, SignalP, LipoP, and PSORT-B all suggested that the proteins are found embedded in the plasma membrane of the cell. They both also lack a cleavage site. The two proteins are, by the evidence provided, likely orthologous ABC transporters. Figure 6 shows the BLAST results.

branched-chain amino acid ABC transporter permease [Meiothermus ruber]

Sequence ID: [WVP_013014899.1](#) Length: 326 Number of Matches: 1

[▶ See 2 more title\(s\)](#)

Range 1: 3 to 326		GenPept	Graphics	▼ Next Match ▲ Previous Match	
Score	Expect	Method	Identities	Positives	Gaps
201 bits(512)	7e-64	Compositional matrix adjust.	133/328(41%)	193/328(58%)	24/328(7%)
Query 1	MSEQFLYFLQQMFNGVTLG	STYALIAIGYTMVYGIIGMINFAHGEVYMIGSYVS---	FMI	57	
	+++ F Q + G+ LG	YA++A+GYTMVYG++G+INFAH EV+MIG+ + F			
Sbjct 3	VADLFATLPQTLLLEGLLLGFVYAMVALGYTMVYGVGLINFAHSEVFMIGAVIGLEVFRF			62	
Query 58	IAALMMMGIDTGWLLVAAGFVGAIVIASAYGWSI	ERVAYRPVRN--SKR-LIALISAIGM		114	
	I ++L+ + A V + +ER AYRP+R SK L+ +I+AI	G+			
Sbjct 63	WGNPESPVIANPFVLLVALIFAAGVSGIMAVLVERFAYRPLRKRGSKNILVPMITAIGV			122	
Query 115	SIFLQNYVSLTEGSRDVALPSLFNGQWVVGHS	EN-----FSASITTMQAVIWI	VTFLAM	168	
	S LQ+ + R + FN Q+ + N F I +I +V+ L +				
Sbjct 123	SFLLQDLTRIYAALRH----NEFNMQYRTYDALNQT	FELPFQTIIQVKGIIIVVSILML		178	
Query 169	LALTIFIRYSRMGRACRACAEDLKMASLLGINT	DRVIALTFVIGAAMA	AVAGVLLGQFYG	228	
	+ LT + +++G+A RA ++D++ ASL+GIN D +I+ TF+IG ++ VAGVL G Y				
Sbjct 179	IGLTYLVNRTKLGAIRAVSQDMQTASLMGINPDV	IISRTFLIGGSLGGVAGVLFGLLYT		238	
Query 229	VINPYIGFMAGMKAFTAAVLGGIGSIPGAMIGGL	ILGIAEALSSAYLS-----TEYK		288	
	+ PY G + G+KAFT+AVLGGIG+IPGAM+GGLILG E LS YL TEYK				
Sbjct 239	NVTPYSGVLPGLKAFTSAVLGGIGNIPGAMVGG	LILGQLETLSGTYLPFLTNGNFGTEYK		298	
Query 281	DVVSFALLILVLLVMPTGILGRPEVEKV		308		
	DV +F L+L+LL P GI G+ EKV				
Sbjct 299	DVFAFLTIVLLLFRPQIFGQNVSEKV		326		

Figure 6: BLAST results from *E.coli* b_3457 BLASTed against *Mrub*_1676 (Madden et al., 2002)

E. coli b_3458 / *Mrub*_1675

Table 5 summarizes the results generated from a variety of bioinformatics tools. The BLAST data shows that the e-value is $8e-52$, which is significantly below the .001 cutoff and suggests that the sequence similarity is likely not by chance. The CDD data, also generated the same COG number, TIGRfam and Pfam all generated the same protein number as well, with very low e-values, which further shows that the two genes are not similar by chance. TMH, SignalP, Lipop, and PSORT-B all suggested that the proteins are found in the cytoplasm, on the interior of the cell. They both also lack a cleavage site. The two proteins are, by the evidence provided, likely orthologous ABC transporters. Figure 7 shows the BLAST results.

branched-chain amino acid ABC transporter substrate-binding protein [Meiothermus ruber]
Sequence ID: [WVP_013014897.1](#) Length: 386 Number of Matches: 1
[▶ See 1 more title\(s\)](#)

Range 1: 24 to 361			GenPept	Graphics	▼ Next Match	▲ Previous Match
Score	Expect	Method	Identities		Positives	Gaps
172 bits(435)	5e-51	Compositional matrix adjust.	121/343(35%)		176/343(51%)	17/343(4%)
Query 26	IKVAVVGAMSGPIAQHGDMFNGARQAIAKDINAKGGIKGDKLVGVEYDDACDPKQAVAVA	85				
Sbjct 24	IKIASVSPLSGPQSGGLGTATIAQGAQMAIEDAQARFQQLGFLQFAPQDDQANPDVGVAVA	83				
Query 86	NKIVND-GIKYVIGHLCSSSTQPASDIYEDEGILMISPGATNPETLQRGVQHIMRTAGLD	144				
Sbjct 84	RRIVNDPDLGLIGVGHLSGVAIASEIYKDTNLVMVSPANTNPRVTDGRLSVNRICGRD	143				
Query 145	SSQGPTAAKYIETVKPQRIAIHDKQQYGEGLARSVQDGLKAANANVFFDGTAGEKD	204				
Sbjct 144	DVQGPVGAIEYAVRIKRSRLFVINDKTPYGGQLAEFAARARELGATVVALVG-TEEASN	202				
Query 205	FSALIARLKKENIDFVYYGGYYPENGQMLRQARSVGLKTQFMGPEGVGNASLSNIAGDAA	264				
Sbjct 203	FQPLILQMRAPDLVYYGGIYDKGGVLVKQMRERGITATFMGGDGLDASDLVKIAGSAS	262				
Query 265	EGMLVTMPK-RYDQDPANQGIVDALKAD-KKDPSPGYVWITYAAVQSLATALE-----	315				
Sbjct 263	KGVLFTTTAGPISTLPKAAAFQRYKAKFGKDPEA-YAVVAYDSANVILAGLEAAIKANN	321				
Query 316	-RTGSDEPLAL-VKDLKANGANTVIGPLNWEKGD LKGFDFGV	356				
Sbjct 322	GRKPTRQVARAVREVKMDG---LTGRIEFDKSGDRKLSDYVV	361				

Figure 6: BLAST results from *E.coli* *b_3458* BLASTed against *Mrub_1675* (Madden et al., 2002)

Why Urea is relevant when discussing ABC transporters.

Urea, is clearly important when discussing ABC transporters, as seen in the results tables. Urea is formed as a result of deamination of amino acids. The same processes that facilitate the transport of amino acids, which are then broken down to components of urea (11).

Conclusion

M.ruber_1675* and *b_3458

M.ruber_1675 and *b_3458* both had the same COG grouping of COG0683. This grouping is the ABC-type branched-chain amino acid transport system, a periplasmic component. The E-value generated for *M.ruber_1675* was 2.42e-46 and for *E. Coli b_3458* it was 2.40e-102. The very low e-value, which is well below the .001 cut off, signals that the data is not generated by chance, and indicates significance. The proteins also had the same Pfam number, which means that they are in the same family in the Pfam database. The both share the PF13458 family, which is the periplasmic binding protein. *M.ruber*'s Pfam E-value was 2.42e-46 and *E.coli* had an E-value of 1.7e-61, the low E-value indicates that these proteins were not placed in the family by random chance. They also had the same TIGRfam hit as well, Tigr03407. *M.ruber* generated a Tigrfam E-value of 1.6e-05, while the *E.coli* generated an E-value of .00042. The cellular Localization signals indicated that both proteins are found in the Cytoplasm, and neither have any transmembrane helices. The next step would be to create a primer that replaces a highly conserved amino acid (Glutamate) into an Alanine and to observe the function. Figure 7 shows the conversion.

Result						
S T V A G K A A L P T E Y S L D R S G Q G R S A N G V Q S R P * R A R P L C Q R S T TCTCGACCGTAGCGGGCAAGGCCGCTCTgccAACGGAGTACAGC AGAGCTGGCATCGCCCGTTCGGCGAGACGGTTGCCTCATGTG						
Required Primers						
Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *	
Q5SDM_2/14/2018_F	AGGCCGCTCTgccAACGGAGTACAGC	26	65	69°C	70°C	
Q5SDM_2/14/2018_R	TGCCCGCTACGGTCGAGA	18	67	72°C		

Figure 7: Substitution of a highly conserved Glutamate at position 15 into an Alanine.

M.ruber_1676* and *b_3457

M.ruber_1676 and *b_3457* both had the same COG groupings as well. Both proteins generated a COG number of 0559, which means they are in the same COG family. This family is the Branched-chain amino acid ABC –type transport system, a permease component. *M.ruber* generated an E-value of $2.39\text{e-}48$ and *E.coli b_3457* generated an E-value of $4.54\text{e-}82$. The very low E-values, well below the .001 cut off, indicate that these proteins are not in this family by chance, and quantify their significance. In addition, Pfam also generated results that showed both the proteins being in the same family, PF02653, which is the branched-chain amino acid transport system, a permease component. The *M.ruber* had an E-value of $6.20\text{e-}18$, and the *E.coli* had an E-value of $6.7\text{e-}71$, both these values are vastly below the minimum cut off, and is indicative of the fact that they were not placed into this family by random chance. TIGRfam also placed the proteins into the same family, they were both in TIGR03410, which is the urea ABC transporter. The *M.ruber* had an E-value of $6.20\text{e-}18$ and the *E.coli* had an E-value of $2.7\text{e-}15$, which are both well below the cutoff, and indicative of their significance. The cellular localization signal indicated that both proteins are found embedded in the membrane, and this is further proven by the fact that each one has multiple transmembrane helices. The next step would be to create a primer that replaces a highly conserved amino acid (Glutamate) into an Alanine and to observe the function. Figure 8

shows the conversion.

Result

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AGCTGGAGGGCCGCATGAgaaATACGCCCTGGTAGCCAG

TCGACCTCCCGCGTACTcttTATGCGGGACCATCGGTC

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
Q5SDM_2/14/2018_F	aATACGCCCTGGTAGCCAG	19	58	68°C	69°C
Q5SDM_2/14/2018_R	tcTCATGCGGCCCTCCAGCT	20	65	72°C	

* Ta (recommended annealing temperature)

Figure 8: The substitution of Glutamate at position 2, to an Alanine.

***M.ruber*_1677 and *b*_3456**

*M.ruber*_1677 and *b*_3456 both had the same COG groupings. Both proteins generated a cog number of 4177. This value belongs to the ABC-type branched-chain amino acid transport system, a permease component. *M.ruber*_1677 had an E-value 7.46e-34 while *E.coli* *b*_3456 had an E-value of 6.05e-78. The low E-values indicate that these proteins were not placed into the family by chance, and that they are significant. Pfam also placed these two proteins in the same family; they were both placed in the PF02653, which is the Amino acid transport system, which is a permease component. *M.ruber* generated an E-value of 4.56e-13 and *E.coli* generated an E-value of 9.0e-60, both values are significantly below the cut off, and are indicative of the fact that they are not placed into the families by random chance. TIGRfam also placed both proteins in the same family. Both were found in the TIGR03410, which is the urea ABC transporter family. Both also had E-values well below the cut off. Both proteins were also found

embedded in the membrane, with multiple transmembrane helices for each one. The next step would be to create a primer that replaces a highly conserved amino acid (Glutamate) into an Alanine and to observe the function. Figure 9 shows the conversion.

Result						
G L A G G V Y A M P R P Y S S P L T M P L R A G R W G L R H A K T L F L S P Y D A F P G W P V G F T P C Q D P I P L P L R C L W CCGGGCTGGCCGGTGGGGTTTACGCCATgccAAGACCCCTATTCCCTCTCCCTTACGATGCCCTTTGG GGCCCGACCGGCCACCCCAATGCGGTACGGTTCTGGGATAAGGAGAGGGGAATGCTACGGAAACC						
Required Primers						
Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *	
Q5SDM_2/14/2018_F	TTTACGCCATgccAAGACCCCTATTCCCTCTCCCTTACGATGCCCTTTGG	48	52	77°C	72°C	
Q5SDM_2/14/2018_R	CCCCACCGGCCAGCCCGG	18	89	81°C		

* Ta (recommended annealing temperature)

Figure 9: Shows the substitution of a highly conserved Glutamate at position 7 into an Alanine.

***M.ruber*_1679 and *b*_3454**

*M.ruber*_1679 and *b*_3454 both had the same COG grouping; they both generated a cog number of 0410. This value belongs to the ABC-type branched-chain amino acid transport system, a ATPase component. *M.ruber*_1679 had an E-value of 2.46e-114 and *E.coli* *b*_3454 had an E-value of 5.11e-136. The low E-values indicate that these proteins were not placed into the family by random chance, and signify their significance. Both proteins also had the same Pfam family, they were both found in the PF00005, which is the ATP-binding domain of ABC transporters. *M.ruber* had an E-value of 2.42e-46 and *E.coli* had an E-value of 3.3e-33, which are both well below the cut off and are indicative that these proteins are not in the PF00005 family by random chance. TIGRfam also showed that both the proteins were in the TIGR03410 family,

which is the Urea ABC transporter, both had very low e-values which is indicative of the fact that the proteins were not placed into the families by random chance. The proteins are also found in the cytoplasm, with no transmembrane helices. The next step would be to create a primer that replaces a highly conserved amino acid (Glutamate) into an Alanine and to observe the function. Figure 10 shows the conversion.

Result					
L P G F V S Q G R Q L F P T W L C K P G A A T F A Y L A L * A R G G N F F GCCTACCTGGCTTTGTAAgccAGGGGCGGCAACTTTTTC CGGATGGACCGAAACATTcggTCCCCGCCGTTGAAAAAG					
Required Primers					
Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
Q5SDM_2/14/2018_F	cAGGGGCGGCAACTTTTTC	19	58	66°C	64°C
Q5SDM_2/14/2018_R	gcTTACAAAGCCAGGTAGGC	20	55	63°C	

* Ta (recommended annealing temperature)

Figure 10: Shows the substitution of a highly conserved Glutamate at position 45, into an alanine.

***M.ruber*_1678 and *b*_3455**

*M.ruber*_1678 and *b*_3455 had different COG groupings. *M.ruber*_1678 had a COG number of 4177 with an E-value of 7.46e-34, while *b*_3455 had a number of 4177 with an e-value of 1.61e-139. The different COG values combined with the low E-values, is indicative that these proteins are not orthologous. In addition, the Pfam results gave different results for the proteins, *M.ruber* was found in the PF00001 family, which is the urea ABC transporter a permease protein. *E.coli* was found in the PF00005, which was the ATP-binding domain of ABC transporters. Both proteins had very low E-values,

which is indicative of the fact that they were not placed in their respective families by random chance. Additionally, they both had different TIGRfam hits, *M.ruber* was placed into the TIGR03408 family while *E.coli* was placed into the TIGR03411 family, which both had very low E-values. Both the proteins were found in the cytoplasm with no transmembrane helices, however. Based on the difference in the bioinformatic tools, it is clear that *M.ruber*_1678 and *b*_3455 are not orthologous. This is not a shock, since they are from different phyla and are likely to have some major differences. The next step would be to create a primer that replaces a highly conserved amino acid (Glutamate) into an Alanine and to observe the function. Figure 11 shows the conversion.

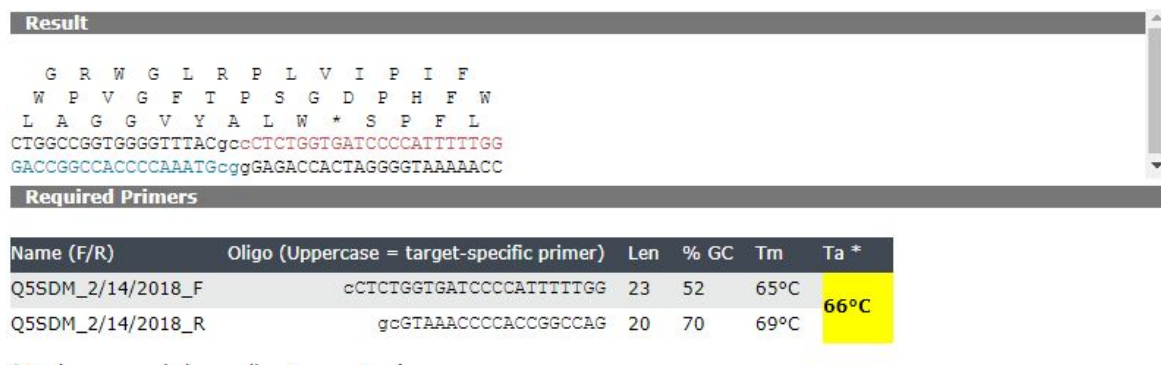


Figure 11: Shows the substitution of a highly conserved Glutamate at position 15 into an alanine

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